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USDA-ARS Western Regional Plant Introduction Station, Washington State University, Pullman, WA, USA

Incidence, Aggressiveness and *In Planta* Interactions of *Botrytis cinerea* and other Filamentous Fungi Quiescent in Grape Berries and Dormant Buds in Central Washington State

F. M. Dugan[1], S. L. Lupien[1] and G. G. Grove[2]

Authors addresses: [1]USDA-ARS Western Regional Plant Introduction Station, Washington State University, Pullman, WA 99164-6402, USA; [2]Department of Plant Pathology, Washington State University IAREC, Prosser, WA 99350-9687, USA (correspondence to F. M. Dugan, E-mail: fdugan@mail.wsu.edu)

With 1 figure

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Abstract

Recovery of quiescent filamentous fungi from non-symptomatic grape berries and dormant buds demonstrated dominance of *Alternaria*, *Aureobasidium*, *Cladosporium*, *Ulocladium* and other dematiaceous hyphomycetes. Up to 78% of berries contained fungi prior to harvest. *Botrytis cinerea* was recovered from 0.2 to 0.5% of surface-disinfested berries just subsequent to fruit set, and 1.6–4.8% of surface-disinfested, over-wintered dormant buds. In laboratory inoculations of mature grape berries with strains of *Alternaria*, *Aureobasidium*, *Cladosporium*, *Ulocladium* and *Botrytis*, only the latter was aggressive in rotting berry fruits. Inoculations with *B. cinerea* alone and in combination with strains of *Alternaria*, *Aureobasidium*, *Cladosporium* and *Ulocladium* recovered from grape demonstrated that prior occupation of wound sites by the latter fungi resulted in reduced lesion size compared to inoculation with *B. cinerea* alone.

Introduction

Endophytic and/or quiescent fungi have been found in several horticultural crops, including grapes (*Vitis vinifera* L.), in which *Botrytis cinerea* is of special interest (McClellan and Hewitt, 1973; Pezez and Pont, 1986; Nair et al., 1995). Invasion by the fungus may occur via the stigma and style, resulting in latent infection of the berry. Later, the fungus may resume growth to induce bunch rot (McClellan and Hewitt, 1973). Under some conditions, such latent infections may account for a high percentage of berry infections (Nair et al., 1995). Sclerotia are the primary means of perennation and the fungus may also over-winter as mycelium in the bark on canes or in dormant buds (Bulit and Dubos, 1988). Infection processes have been reviewed (Coertze et al., 2001).

B. cinerea is a primary target of standard vineyard management practices in Washington and the Pacific Northwest (Grove, 1998; Pscheidt, 1999; Pscheidt and Ocamb, 2000). The degree to which the fungus exists as quiescent infections in *Vitis* berries or other plant parts in the prime vineyard country of south-central Washington has not been previously reported. Our primary objectives were to ascertain the degree to which *B. cinerea* or other fungi occur as quiescent infections in non-symptomatic grape berries, and to determine the frequency with which non-symptomatic, dormant buds contain *B. cinerea*. Subsidiary objectives were to determine if common vineyard fungi, dematiaceous hyphomycetes other than *B. cinerea*, would rot berries more slowly than *B. cinerea*, and to see if such fungi might exhibit an inhibiting effect against *B. cinerea* when established in wounds prior to challenge with *B. cinerea*.

Materials and Methods

Harvesting sites and dates

Fifty White Riesling grape berries of approximately stage 29 (Eichhorn and Lorenz, 1977) were harvested from each of eight vines (total of 400 berries) near Prosser, WA (site 'A'), on 29 July 1999, and the procedure was repeated for berries at approximately stages 31–33 in the same plots on 30 August 1999. On 3 March 2000, three dormant, over-wintering buds were harvested from each of 144 vines (total of 432 buds) in the same plots. On 7 July 2000, 50 Chardonnay grape berries of approximately stages 27–29 were taken from each of 12 vines (total of 600 berries) on a second set of plots (site 'B') in the same locale. On 9 September 2000, 10 berries of approximately stage 33 were taken from each of 12 vines (total of 120 berries) of the same plots. On 29 March 2001, 20 over-wintering dormant buds of cultivars Chardonnay or White Riesling were

taken from each of 30 vines (total of 300 buds from each cultivar) in a third plot near Prosser, WA (site 'C'). None of the samples were to be from vines subjected to fungicides, except for applications of Rubigan (fenarimol, 12% a.i., at 420 g/ha) at sites 'A' and 'B'. A sample of 200 berries (approximately stage 27) from 24 June 1999 at site 'A' was later discovered to be from vines treated with anti-*Botrytis* fungicidal sprays, so on 29 June 2001 an additional spring sample, 15 berries (approximately stage 29) from each of 30 vines (total of 225 berries from each cultivar), was taken at site 'C'.

Processing of berries and dormant buds and recovery of fungi

Healthy looking berries and buds were excised from the plant with a scalpel disinfested by dipping in 0.5% NaOCl or 70% ethanol between cuts, then placed in clean plastic bags on ice and transported to the laboratory. Prior to plating onto semi-selective or non-selective media, berries and buds were surface-disinfested by immersion for 30 s in 70% ethanol, followed by 5 min in 0.5% NaOCl to which one drop of Tween 20 per 500 ml had been added, then rinsed with sterile, distilled water. Previous tests with conidial suspensions of *Alternaria* and *Cladosporium* strains confirmed the ability of these solutions to kill conidia. Berries >5 mm diameter (approximately stage 33) were bisected prior to plating. Standard aseptic procedures were applied throughout the isolation process. Media employed for the samples taken through 26 September 2000 were Difco malt extract agar (MEA) amended with 50 mg/l streptomycin sulphate and 50 mg/l tetracycline hydrocloride after autoclaving (antibiotic MEA), or, in a third of the plates, with rose bengal at 35 mg/l (rose bengal MEA). The latter retards growth of all fungi and enhances recovery of slow-growing isolates. For samples subsequent to 26 September 2000, antibiotic MEA was supplemented with media semi-selective for *Botrytis* (modified from Kritzman and Netzer, 1978): half strength V8 agar ($\frac{1}{2}$V8) (Stevens, 1981) or Difco potato dextrose agar amended with 5 g/l tannic acid, 8.2 mg/l pentachloronitrobenzene (PCNB) (Terraclor 75% WP, Olin Corp., Norwalk CA, USA), 0.92 mg/l mancozeb (Penncozeb 75% WP, Rohm and Haas Co., PA, USA), and 100 mg/l streptomycin sulphate (all added after autoclaving) and adjusted to pH 4.6–5 with 1 M HCl. One-third of these latter samples were plated to antibiotic MEA, and two-thirds to the semi-selective media.

Identification and preservation of fungi

Filamentous fungi were transferred from isolation plates to $\frac{1}{2}$V8, $\frac{1}{2}$V8 amended with autoclaved or radiation-sterilized plant materials, or MEA and incubated at ambient laboratory temperature (approximately 22–26°C) under a combination of fluorescent and near-ultraviolet (black) lights to induce sporulation. Representative strains were selected and identified using cultural and morphological criteria from Shoemaker (1964), Barron (1968), Simmons (1969, 1985, 1986, 1990, 1994, 1995), Ellis (1971, 1976), Malloch and Cain (1973), von Arx (1975), Hermanides-Nijhof (1977), Carmichael et al. (1980), Sutton (1980), Hawksworth and Yip (1981), Walker and Minter (1981), Klich and Pitt (1988), Petrini (1992), Nag Raj (1993), de Hoog and Yurolova (1994), Bell and Mahoney (1995), Chaisrisook et al. (1995), Andersen and Thrane (1996), Ellis and Ellis (1997), Ho et al. (1999), Kiffer and Morelet (1999) and Pitt (2000). Fungal isolates were preserved with silica gel, in liquid nitrogen vapour, in 15% glycerol at 80°C, or on agar slants under mineral oil (Smith and Onions, 1994). For the 29 March 2001 and 29 June 2001 samples, only *Botrytis* and taxa not previously isolated in the study were preserved.

Inoculation of mature grape berries with single strains

Strains used were isolated from asymptomatic grape berries during the previous portions of the study: *Alternaria alternata* (W99-256), *Alternata infectoria* (WF99-55), *Aureobasidium pullulans* (WF99-126, W99-30), *B. cinerea* (R1V55, 101V3Dd), *Cladosporium herbarum* (WF99-31, W99-325), *Cladosporium cladosporioides* (WF99-47, W99-175b) and *Ulocladium atrum* (WF99-129, W99-385). Mature grape berries (organically grown Chardonnay, approximately stage 35) were disinfested in 75% ethanol for 30 s followed by 5 min in 0.5% NaOCl and a sterile distilled water rinse. Berries were wounded by piercing once the epidermis to a depth of 2 mm with a sharp, sterile needle. Five berries were inoculated with a given strain in each of two replications. Suspensions of conidia were prepared by harvesting conidia from $\frac{1}{2}$V8 into sterile distilled water, filtering through sterile cheese cloth, and adjusting the concentration to 10^4 conidia/ml prior to placement of one drop (ca. 40 μl) of inoculum over the wound site. Ten berries, five per replication, similarly wounded but inoculated only with distilled water, served as controls. Berries were placed in disinfested (1.0% NaOCl + distilled water rinse) plastic racks in which berries were individually separated. Racks were enclosed in plastic bins over a thin layer of distilled water to prevent desiccation and incubated at approximately 22–26°C for 10 days prior to recording lesion diameters with a Max-Cal digital caliper (SLS-LUX Scientific, Inc., Millville, NJ, USA). Results were analysed via General Linear Model (GLM) in SAS version 6.12 (SAS Institute Inc., Cary, NC, USA) for detection of differences of aggressiveness within and between species. The experiment was repeated once.

Inoculation of mature grape berries with strains, followed by inoculation with *B. cinerea*

In a separate experiment, each of the above strains other than *B. cinerea* was used to similarly inoculate 10 berries (five per replication) 3–4 days prior to challenge of the same wounds with 10^4 conidia/ml of *B. cinerea* strain 101V3Dd. Another 10 (five per replication) were similarly wounded but inoculated only with distilled water prior to challenge. After challenge

Table 1
Seasonal colonization of grape berries by fungi

Date	Site	Number of berries in sample	Percentage of berries colonized	Number of isolates recovered
06/24/99	A	200	6	12
07/30/99	A	400	14	62
08/30/99	A	400	25	106
07/03/00	B	600	50	322
09/26/00	B	120	78	119
06/29/01	C	450	66	338[1]

[1] Only fungi of special interest were recovered into pure culture in 2001, but isolates were identified to genus on the basis of colony and morphological characters (Table 2).

Table 2
Relative dominance (%) of fungal taxa in grape berries by site and sampling date

Date	06/24/99[1]	07/30/99	08/30/99	07/03/00	09/26/00	06/29/01
Site	A	A	A	B	B	C
% *Alternaria*	33	10	17	62	80	58
% *Cladosporium*	25	24	31	21	13	21
% *Aureobasidium*	0	5	5	<1	5	<1
% *Ulocladium*	0	10	11	3	0	4
% Other hyphomycetes	9	11	8	2	1	1
% Coelomycetes	9	4	2	1	0	<1
% Ascomycetes	8	5	4	<1	0	<1
% Yeasts	0	8	8	<1	0	<1
% *B. cinerea*	0[1]	0	0	1	0	<1
% Non-sporulating	16	23	16	9	1	11
Total	100	100	100	99	100	100

[1] The 06/99 sample was compromised with regard to recovery of *B. cinerea* because of fungicidal application.

with *B. cinerea*, berries were incubated as before for 25 days at which time lesion diameters were recorded. Berries and equipment were disinfested as in the previous set of experiments. The experiment was repeated and analysed with GLM as before.

Results

Fungi quiescent in asymptomatic berries

Grape berries were progressively infected with quiescent fungi as the season progressed. The percentage of berries colonized just prior to harvest varied from 25 to 78% while infection rates just subsequent to fruit set ranged from 6 to 66% (Table 1). Dominant genera of fungi quiescent in berries were *Alternaria*, *Cladosporium*, *Aureobasidium*, and *Ulocladium* (Table 2). Most fungal growth from disinfested berries originated from the base of the attached stem or from the stylar scar. *B. cinerea* was recovered from 0.50% of young, set fruit in July 2000, and from 0.22% of similar fruit in June 2001. *B. cinerea* represented 0.95% of the recovered strains in July 2000, and 0.30% in June 2001 (Table 2).

The following fungi were recovered from non-symptomatic berries: *A. alternata* (Fr. : Fr.) Keissler, *Alternaria infectoria* Simmons, *Alternaria tenuissima* (Fr.) Wilshire, *Aspergillus fumigatus* Fresen., *Aspergillus niger* Tiegh., *Au. pullulans* (de Bary) G. Arnaud, *B. cinerea* Pers. : Fr., *Chaetomium globosum* Kunze : Fr., *Chaetomium* sp., *C. cladosporioides* (Fresen.) G.A. de Vries, *C. herbarum* (Pers. : Fr.) Link, *C. herbarum* (Pers.) Link var. *macrocarpum* M.H.-M. Ho & F.M. Dugan, *Cladosporium* sp., *Epicoccum nigrum* Link, *Gonatobotrys simplex* Cda., *Idriella lunata* P.E. Nelson & K. Wilh., *Microsphaeropsis olivacea* (Bonard.) Höhn., *Penicillium olsonii* Bainier & Sartory, *Penicillium* sp., *Phialophora* sp., *Phoma* sp., *Podospora tetraspora* (Winter) Cain, *Rosellinia* cf. *limoniispora*, *Sepedonium* sp., *Sporothrix* sp., *Stemphylium herbarum* Simmons, *Stemphylium vesicarium* (Wallr.) Simmons, *Stemphylium* sp., *Thielavia terricola* (Gilman & Abbott) Emmons, *Thielavia* sp., *Torula herbarum* (Pers. : Fr.) Link, *Truncatella angustata* (Pers.) S.J. Hughes, and *U. atrum* G. Preuss. In addition, miscellaneous unidentified coelomycetous and yeast strains were recovered (Table 2). Identification of yeasts will form the subject of separate research.

Fungi quiescent in over-wintered dormant buds

Eighty per cent of the buds from site 'A' produced fungi when disinfested and incubated on non-selective media, and *B. cinerea* was recovered from 1.6% of these buds. All buds from site 'C' produced fungi when disinfested and plated to non-selective and semi-selective media, and 4.8% of the buds produced *B. cinerea* (Table 3). Dominant genera were *Alternaria*, *Cladosporium*, *Aureobasidium* and *Ulocladium*, but at site 'A' *Sordaria fimicola* accounted for 8% and *Rosellinia* cf. *limoniispora* for 6% of strains recovered. At site 'C', *Gonatobotrys* accounted for 2% of strains recovered; *Epicoccum* and *Epicoccum*-like colonies accounted for an additional 2% of the other hyphomycetes. *B. cinerea* accounted for 2% of recovered strains at site 'A' and 3% at site 'C' (Table 4).

Table 3
Percentage colonization of overwintered dormant grape buds by fungi

Date	Site	Number of buds in sample	Percentage of buds colonized by fungi	Total number of fungal strains recovered	Percentage of buds colonized by *Botrytis*
03/03/00	A	432	80	418	1.6
03/29/01	C	600	100	1141	4.8

Table 4
Relative dominance of fungal taxa in grape dormant buds by site and sampling date

Date Site	03/03/00 A	03/29/01 C
% *Alternaria*	37	53
% *Cladosporium*	7	30
% *Aureobasidium*	13	1
% *Ulocladium*	8	4
% Other hyphomycetes	4	6[1]
% Coelomycetes	1	<1
% Ascomycetes	17[2]	<1
% Yeasts	2	<1
% *B. cinerea*	2	3
% Non-sporulating	9	4
Total	100	100

[1] Principally *Gonatobotrys simplex* and *Epicoccum nigrum*.
[2] Principally *Sordaria fimicola* and *Rosellinia* cf. *limoniispora*.

The following fungi were recovered from non-symptomatic, dormant buds: *Acremonium sclerotigenum* (F. & R. Moreau ex Valenta) W. Gams, *Acremonium* sp., *A. alternata*, *A. infectoria*, *A. tenuissima*, *Au. pullulans*, *B. cinerea*, *C. cladosporioides*, *C. herbarum*, *C. herbarum* var. *macrocarpum*, *Cladosporium malorum* Ruehle, *Coniochaeta* cf. *leucoplaca*, *Coniochaeta* cf. *ligniaria*, *Coniothyrium* sp., *E. nigrum*, *Fusarium* sp., *G. simplex*, *Lecythophora* sp., *Penicillium aurantiogriseum* Dierckx, *Penicillium chrysogenum* Thom, *Penicillium oxalicum* Currie & Thom, *Phoma* sp., *Pruessia* sp., *Rosellinia* cf. *limoniispora*, *S. fimicola* (Roberge ex Desmaz.) Ces. & De Not., *Diplodia mutila* (Fr.) Mont., *Sporothrix* sp., *S. herbarum*, *S. vesicarium*, *T. terricola*, *T. angustata*, *U. atrum* and *Ulocladium* cf. *consortiale*. In addition, miscellaneous coelomycetes and yeasts were recovered (Table 4).

The same genera of dematiaceous hyphomycetes (viz. *Alternaria*, *Aureobasidium*, *Cladosporium* and *Ulocladium*) were strongly represented regardless of tissue (berries vs. buds), year or host cultivar.

Inoculation of mature grape berries with single strains

The effects for species and strain on lesion size were significant. Mean lesion size of 5.4 mm for *B. cinerea* differed from all others at $P \leq 0.05$. The next largest mean, 2.1 mm, was for *A. alternata*, which differed at $P \leq 0.05$ only from *B. cinerea* and the control (1.0 mm). All other means (1.6–1.9 mm) were not significantly different from each other or from the control. When the experiment was repeated, a mean lesion size of 6.0 mm for *B. cinerea* differed from all others at $P \leq 0.05$. The next largest mean, 3.2 mm, again for *A. alternata*, differed at $P \leq 0.05$ from *B. cinerea* and all other means (1.7–2.1 mm), which did not significantly differ from each other or from the control (1.2 mm).

With respect to strain, *B. cinerea* 101V3Dd (mean lesion 7.6 mm) and R1V55 (mean lesion 3.3 mm) differed from each other and from all others at $P \leq 0.05$, but *A. alternata* W99-256 (mean 2.1) failed to differ ($P \leq 0.05$) from all remaining means (1.4–2.1) other than the control (1.0 mm). When the experiment was repeated, *B. cinerea* 101V3Dd (mean lesion 7.6 mm) and R1V55 (mean lesion 4.5 mm) again differed from each other and from all others at $P \leq 0.05$. *A. alternata* W99-256 (mean 3.2 mm) failed to differ from *Au. pullulans* WF99-126 and *Au. pullulans* W99-30 (means 2.1 mm) but differed from the remaining strains (mean 1.6–2.0 mm) and the control (mean 1.2 mm). All strains except 101V3Dd, R1V55 and W99-256 failed to differ from each other and the control. Figure 1 represents pooled results for both experiments.

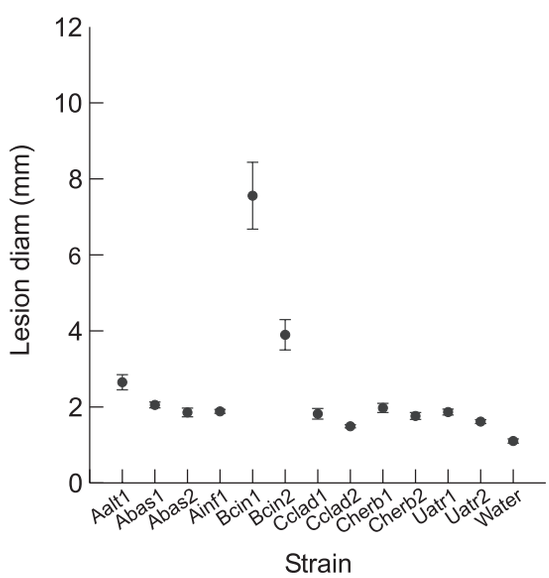

Fig. 1 Lesion diameters induced by inoculation of mature Chardonnay grape berries with fungal strains. Combined results for two experiments. Aalt1 = *Alternaria alternata* W99-256; Abas1 = *Aureobasidium pullulans* W99-30; Abas2 = *A. pullulans* WF99-126; Ainf1 = *Alternaria infectoria* WF99-55; Bcin1 = *Botrytis cinerea* 101V3Dd; Bcin2 = *B. cinerea* R1V55#13; Cclad1 = *Cladosporium cladosporioides* W99-175B; Cclad2 = *C. cladosporioides* WF99-47; Cherb1 = *Cladosporium herbarum*. WF99-31; Cherb2 = *C. herbarum*. W99-325; Uatr1 = *Ulocladium atrum*. WF99-129; Uatr2 = *U. atrum*. W99-385; water = water control. Bars are standard errors

Inoculation of mature grape berries with strains, followed by inoculation with *B. cinerea*

Lesions resulting from inoculation of wounds with *B. cinerea* alone produced the highest mean of 7.3 mm, which differed ($P \leq 0.5$) from all other means (2.8–4.8 mm). These other means did not differ amongst themselves, except that the second highest (4.8 mm for prior inoculation with *C. hebarum*) differed from the lowest (2.8 mm for prior inoculation with *U. atrum*). When the experiment was repeated, the highest mean (9.4 mm, for inoculation with *B. cinerea* in the absence of prior inoculation) differed ($P \leq 0.05$) from all other means (2.7–4.5 mm) which did not significantly differ amongst themselves.

Discussion

Our studies demonstrate that quiescent infections of grape berries and dormant buds are common in south-central Washington state, and that the total frequency of such infections (because of several fungal species) increases over the growing season (Tables 1 and 2). Latent infections from *B. cinerea* are established in immature berries, and *B. cinerea* over-winters within bud tissues in south-central Washington.

That the percentage of fruits bearing quiescent infections increases with time agrees with research on other fruit crops (Dugan and Roberts, 1994; Serdani et al., 1998). McClellan and Hewitt (1973) reported growth of fungi other than *B. cinerea* from over 60% of surface-disinfested grape berries, results similar to our own. But in some instances, the proportions of *Vitis* berries containing latent infections can be considerably higher than reported in our study: Gindrat and Pezet (1994) reported instances of latency with nearly 70% infection from *B. cinerea* alone in the region d'Epernay in France. In McClellan and Hewitt (1973), the proportion of berries with quiescent infections because of fungi other than *Botrytis* increased over the growing season, but the proportion of latent infections because of *B. cinerea* decreased as the crop matured. We detected latent *Botrytis* infections only early in the season. As bunch rot was later visible throughout our plots, it is plausible that these latent infections progressed to bunch rot over time. The implication is that *B. cinerea* becomes an aggressive pathogen as the grape approaches maturity, but that other fungi are less aggressive or remain quiescent. Our own results with inoculations of berries in the laboratory confirmed that *B. cinerea* is more aggressive than other common vineyard fungi from south-central Washington. There is a substantial literature on *B. cinerea* and wine quality, but analogous effects for other filamentous fungi are little examined (Fleet and Heard, 1993).

The taxa of fungi endophytic in *Vitis* leaves, petioles, peduncles or nodes may differ from the taxa reported here. Mostert et al. (2000), collecting material from South African vineyards, recovered *Phomopsis viticola* as well as numerous other species not recovered in our studies. However, *Alternaria*, *Epicoccum*, *Cladosporium*, *Ulocladium*, and *Sordaria* were recovered by them, so the fungal floras of the two studies overlap considerably in spite of differences in geographic locale and sampled plant parts. Cardinale et al. (1994) also concentrated on foliage and recovery of *P. viticola*. They also reported *Alternaria*, *Epicoccum* and *Cladosporium*. Unlike Schweigkofler and Prillinger (1997) we did not isolate *Verticillium dahliae*.

The relatively dry climatic conditions in south-central Washington probably mitigate the degree to which latent *B. cinerea* infections are established in the berries; hence, the relatively low numbers in our studies compared to results in Gindrat and Pezet (1994). Latent infections have been demonstrated to initiate via the floral parts (McClellan and Hewitt, 1973). Nair et al. (1995) have quantified the relation between carry-over inoculum (conidia produced from mummified berries) and subsequent infection of flowers and berries under conditions prevalent in New South Wales, but the relation between infection of over-wintering dormant buds and subsequent flower and berry infections, quiescent or otherwise, is not known. Sources of inoculum from over-wintered dormant buds may be of diminished importance relative to inoculum from sclerotia (Nair and Nadtotchei, 1987). Similarly, there may be situations in which colonization of floral debris in tight berry clusters (Northover, 1987) may outweigh the importance of quiescent infections. The capacity of *Botrytis* to invade hosts from senescent floral debris is well established for a number of crops, as is the tendency to defer attack on host fruit tissues until ripening commences (Verhoeff, 1980). Jarvis (1977) lists the various agents (weather, insects, mildew, etc.) capable of inflicting injuries predisposing plants to invasion by *Botrytis*. Although our studies are currently insufficient in duration and extent of sampling for accurately quantifying the effects of quiescent infections on the epidemiology of bunch rot in south-central Washington, they nonetheless clearly demonstrate the potential for impact. Approximately one in 200 young, spring berries demonstrated quiescent infection with *Botrytis*, which implies that a fairly high proportion of grape bunches could contain such infections.

As *B. cinerea* is a cosmopolitan fungus, its probable occurrence in dormant buds of distributed vegetative germplasm may have minimal significance. However, there are indications that variability in population structure, fungicide resistance and aggressiveness of *B. cinerea* in vineyards may be greater than previously thought (Donèche, 1993; Giraud et al., 1999). The two strains used in our study differed in aggressiveness. Strains of *B. cinerea* with cross-resistance to fungicides are of concern in grape production (e.g. Leroux et al., 1999). As *Vitis* germplasm is routinely distributed as untreated cane cuttings (Warren Lamboy, Curator, USDA-ARS NERPIS, pers. commun.) the potential for spread of aggressive *Botrytis* strains should be noted. In this same regard, we mention our single isolation of *D. mutila*, agent of black dead arm of grape, from a non-symptomatic bud. *D. mutila*

(teleomorph = *Botryosphaeria stevensii* Shoem. = *Physalospora mutila* Stevens) is present in Washington state (Farr et al., 1989), but black dead arm is not presently documented from North America (Common Names of Plant Diseases: www.apsnet.org).

Given that recovery of *B. cinerea* from non-symptomatic berries occurred only in the spring samples and not in samples from later in the season, and given that total numbers of quiescent infections by fungi other than *B. cinerea* increased with time over the growing season, we propose the hypothesis that under conditions in south-central Washington, niche competition from common dematiaceous hyphomycetes preempts much available substrate and thereby constrains the number of quiescent infections from *Botrytis*. Additional indirect evidence in favour of this hypothesis is the competitive ability of common saprophytic fungi inoculated into mature fruits: in our studies, colonization of artificial wounds with any one of several common vineyard fungi resulted in reduced lesion formation compared to situations in which wounds were not so colonized prior to inoculation with *Botrytis*. Provided that the crop in question is destined for wine production (i.e. rapid processing) and not for prolonged refrigerated storage and distribution such as characterizes table grapes, verification of this hypothesis would constitute a strong rationale for use of fungicides specific to *Botrytis*, and with less impact on its common saprophytic competitors. Some of the fungi isolated in our study (notably *Alternaria*, *Cladosporium* and *Penicillium*) are known post-harvest pathogens of grape (Snowdon, 1990) and are documented from central Washington (Sprague, 1953), but others, (*Aureobasidium* and *Ulocladium*), have been used for experimental biological control of *Botrytis* (Dik and Elad, 1999; Dik et al., 1999; Kessel et al., 1999; Köhl et al., 1999). Such common fungi, although capable of rotting mature grapes given sufficient time, may act beneficially by retarding the establishment of gray mould in the field and thus might be exploited as allies against *B. cinerea*.

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